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DRUG DISCOVERY

Rice husk as a sustainable source of microcrystalline cellulose: pharmacopoeial, crystalline and spectroscopic characteristics

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ABSTRACT

Herein, we report the use of three methods to pulp rice husks to find a method that results in near lignin-free pulp. Using dilute hydrochloric acid, the near lignin-free pulp (α-cellulose) was depolymerized into microcrystalline cellulose (RH-MCC).RH-MCC was examined for pharmacopoeial and some critical technical characteristics using X-ray powder diffractometer, FT-IR and NMR. We found that RH-MCC met the official specifications for microcrystalline cellulose (USP); the spectroscopic analysis further showed that

RH-MCC is lignin-free and its calculated crystallinity index, which is often a reflection of its ability to form compact, is 50%, and its polymorphic form is cellulose II. In view of the above, the RH-MCC powder derived from rice husks is a potential pharmaceutical excipient.

Keywords: Microcrystalline cellulose, Pharmacopoeia characteristics, Agricultural residue, Biomass, Rice husk.

1. INTRODUCTION

Microcrystalline cellulose (MCC) is a multifunctional excipient that finds applications in pharmaceutical, food, cosmetic, and medical industries as a suspension stabilizer, a water-retainer, and as a diluent and flow-aid in pharmaceutical tableting. Traditionally, MCC used in the industries is obtained by acid hydrolysis of wood and purified cotton. These sources are expensive; hence there has been continued research for alternative feedstock for production of MCC (El-Sakhawy and Hassan, 2007).

The utilization of non-wood sources of fibers, as alternative feeds, in production of pulp for industrial applications has received heightened attention. This is because of two main reasons; firstly, the decline in forest resources due to deforestation across the globe; secondly, agricultural residues provide excellent alternative materials because they are in abundance, renewable, inexpensive, and their utilizations have advantages of economy (second income to farmers), environment (reduced air pollution generated from their incineration) and technology (Ohwoavworhua et al., 2009). It is against the above background that a number of investigators have examined agricultural wastes and other sources besides cotton and wood-trees for cellulose. Few of such studies include, but not limited to, corn stover (Costa et al., 2015), corn cobs (Azubuike and Okhamafe, 2012), groundnut husks (Ohwoavworhua et al., 2009), cassava peel (Widiarto et al., 2017), wheat straw and soy hulls (Alemdar and Sain, 2008), rice straw, bagasse and cotton stalks (El-Sakhawy and Hassan, 2007), coconut shells (Ohwoavworhua et al., 2006), and native sponge, *Luffa cylindrical* (Ohwoavworhua et al., 2004).

Rice (obtained from *Oriza sativa* plant) is one of the most important cereal products worldwide. Its yearly production, globally, is estimated at 741.5 million tons of rice paddy (or rough rice). Processing of paddy rice yields 70% white rice, 20% rice husks, 8% rice bran, and 2% rice germ (Kumar et al., 2012). Rice husks (also called rice hulls) as part of chaff from rice production therefore exist as huge agricultural waste. Liu et al., (2013) reported that for every five tons of rice harvest, one ton of husks waste is generated – amounting to 1.72 $\times 10^8$ tons of rice per year, globally. Rice husk contains cellulose, lignin, silica, alkalis, and trace elements – all totaling 75-90% of the organic matter (Kumar et al., 2012). In most countries where rice is produced, much of the husks are either burnt or dumped as waste (Kumar et al., 2012); thus resulting in not only serious pollution of the environment, but a huge waste of resources. Few uses of this organic waste material include it use as building material, fertilizer, insulation material, formation of activation carbon, or fuel (Kumar et al., 2012).

Most recently however, rice husks waste has been investigated as a source of bio-oils via pyrolysis processing (Huang et al., 2018; Alvarez et al., 2014), and even as source of hydrogen production, which is through steam reforming of bio-oils at an elevated temperature with nickel-based catalyst (Chen et al., 2011). Also, it has been investigated as a sustainable source of nano structured silicon for high performance lithium ion battery anode (Liu et al., 2013). These processes are thermochemical conversion of rice husks biomass, which is usually very costly because of the demands of high temperature processing, besides the generation of substantial quantities of air pollutants (Abbasi and Abbasi, 2010).

In this study, we process rice husks to obtain a lignin-free pulp, and the pulp was acid hydrolyzed into microcrystalline cellulose that was evaluated for pharmacopoeia and some critical technical attributes, with a view to assess its usability as a pharmaceutical excipient.

2. MATERIALS AND METHODS

Materials

The materials requirement for this work include nitric acid, sodium nitrite, sodium sulphite, sodium hydroxide phosphoric acid, hydrochloric acid, sulphuric acid, acetic acid, formic acid, dimethyl sulphur oxide (DMSO), toluene, ethanol, Avicel® PH 101 (particle size, ~ 50 µm) (FMC Corporation, USA), xylene, phloroglucinol and iodine crystals were supplied by Sigma-Aldrich, UK and used as obtained. Sodium hypochlorite as JIK® was supplied by Reckitt and Colman Ltd., Nigeria. The water was double distilled and all other chemical used was of analytical reagent grade. Rice husks sample was obtained from a local mill, washed with water, and used as obtained, without further milling.

Extraction of α-cellulose

Pre-treatment of agricultural residues

The rice husk sample was washed in tap water to free it of all extraneous materials and dried at room temperature in a dust-free air before being used for the pulping procedures. Two major types of pulping methods were used, as follows:

Inorganic pulping

Sodium hydroxide pulping

Previously reported method was used with sight modifications (Ohwoavworhua et al., 2004). Briefly, the pre-treated rice husks residue (350 g) was placed in a *Pyrex* glass vessel to which was added 3 L of 4 % w/v sodium hydroxide and allowed to digestion for 2 h at 80 ^{0}C in a water bath (FGL 1083 Karl Kolb Scientific). This step converts rice husks lignin into water-soluble complexes and are removed. Following thorough washing and filtration, it was bleached (thrice) with 2.0 L of a 1:1 aqueous dilution of sodium hypochlorite for 40 min at 80^{o}C. The washed and filtered material was then treated with 2.0 L of 17.5 % w/v sodium hydroxide at 80 ^{o}C for 1 h. The resulting α-cellulose was rinsed thoroughly with water. The resulting pulp was treated to a whitening step using a 1:1 aqueous dilution of sodium hypochlorite for 20 minutes at 80 ^{o}C and was washed until it is neutral to litmus paper. The cellulose material was filtered, pressed, manually reduced to small lumps and dried in a fluidized bed dryer (Copley), at temperature of 57 – 60 ^{o}C for 60 min.

Nitric acid pulping

The method of Azubuike and Okhamafe (2012), with a slight modification, was used. A 350 g pre-treated rice husks sample was immersed in solution (3 L) of 3.5% nitric acid containing 40 mg of sodium nitrite for 2 h in a *Pyrex* glass container that was placed in a water bath (FGL 1083 Karl Kolb Scientific) set at 90 ^{o}C to remove lignin in the form of soluble nitro lignins. Following thorough washing and filtration, it was digested with a 2.5 L solution containing 2 % $^w/_v$ each of sodium hydroxide and sodium sulphite at a temperature of 50 ^{o}C for 1 h. Again, it was washed, filtered and bleached with a 2 L 1:1 aqueous dilution of 3.5 %$^w/_v$ sodium hypochlorite at boiling temperature for 20 min. The washed and filtered material (i.e., holocellulose) was next treated with 1.5 L of 17.5 %$^w/_v$ sodium hydroxide at 80 ^{o}C for 0.5 h. The resulting α-cellulose was washed thoroughly with water. Bleaching in an aqueous dilution of 1.75 % w/v sodium hypochlorite for 5 min at 100 ^{o}C and subsequent washing with water until the filtrate was clear then completed the extraction process. The cellulose material was filtered, and the water manually squeezed out to obtain small lumps, which were dried at 60^{o}C in fluidized bed dryer.

Organosolv pulping

Formic acid-acetic acid-water delignification

The method reported by Sun et al., (2004) was used, with slight modifications. Briefly, 200 g of the pretreated rice husks sample was placed in a *Pyrex* glass container and 2.5 L of a solvent mixture of formic acid-acetic acid-water (3:6:1). Digestion was effected for 4 h at 85 ^{o}C in a water bath while using hydrochloric acid (0.1%) as a catalyst. Following thorough washing with water and filtration, the residue was bleached in 1.2 L of 1.75 %$^w/_v$ aqueous dilution of sodium hypochlorite for 30 min at 100 ^{o}C. It was washed and then further whitening with a 1.75 %$^w/_v$ aqueous dilution of sodium hypochlorite for 15 min at 80 ^{o}C and washed with water until neutral. The cellulose material was filtered and pressed in a calico cloth, and manually reduced to small lumps and then dried in a fluidized bed dryer (Copley), at an inlet air temperature of 57 – 60 ^{o}C for 60 min.

Compositional properties of obtained pulps

Klason lignin determination

The method reported by Viera et al., (2007) was used. Briefly, a 2 g sample of the pulp was transferred to a round bottom flask and 15 ml of sulphuric acid (72 %) was slowly added to it under stirring. The sample was stirred in a bath at room temperatures for 2 h. Subsequently, 560 ml of distilled water was added to the round-bottomed flask, diluting the acid solution to 3 %. The system was heated up, under reflux, boiling, left to stand to allow the insoluble residue to sediment, filtered through a previously weighed sintered crucible, and washed with 500 ml of distilled hot water. The residue was dried in an oven for 12 h at 105 ^{o}C. The dry residue, lignin, was weighed and expressed as % lignin. The data are shown in Table 2.

Determination of α-cellulose content

The determination of α-cellulose was carried out as follows: Twenty five ml of 17.5% sodium hydroxide solution was added to 3 g of pulp. The slurry, after being left to swell for 4 minutes at 20 ^{o}C was pressed for 3 minutes with a glass rod, and another 25 ml of 17.5

% sodium hydroxide solution was added. The suspension was thoroughly mixed for 1 min and left covered at room temperature. After 35 min, 100 ml of distilled water were added, followed by filtration in a sintered glass crucible. The filtrate was poured twice on the paste before washing with distilled water till complete neutrality was achieved. Acetic acid (10 %, 50 ml) was added followed by distilled water. The pulp was finally dried at 105 ^oC for 5 h and weighed (Viera et al., 2007). The % residue (i.e. α-cellulose) data are recorded in Table 2.

Preparation of microcrystalline cellulose (MCCs)

The procedure reported earlier (Ohwavworhua et al., 2004), with a slight modification, was used. A 100 g quantity of the α-cellulose obtained from the rice husks sample was placed in a *Pyrex* glass beaker of 2 L capacity and hydrolyzed in 2.0 N hydrochloric acid (1.2 L), and at boiling temperature for 15 min. The hot acid mixture was poured into cold tap water, vigorously stirred with a spatula and allowed to stand overnight. The microcrystalline cellulose (code named as RH-MCC) obtained by this process was washed with water until neutral, pressed and dried in a fluidized bed dryer at a temperature of 57 – 60 ^oC for 60 min. Following size reduction (using Kenwood blender) and sieving, the fraction passing through a 0.710 mm sieve aperture was obtained and stored at room temperature in an air-tight container. The physicochemical and powder characteristics of the MCCs were evaluated as described below.

Pharmacopoeial attributes of RH-MCCs

The compendia specifications of RH-MCC such as identification, solubility, water-soluble substances, starch and dextrin, organic impurities, loss on drying, pH, and total ash determinations were determined in accordance with USP37-NF32, 2014. All physiochemical determinations were carried out in triplicate and the means recorded in Table 2.

X-ray powder diffractometer (XRD) determination

Diffraction pattern was measured for RH-MCC sample using Phillips X-ray diffractometer. The diffraction pattern was recorded using Cu-Kα radiation at 40 kV and 25 Ma. The sample was pressed into pellets (25mm in diameter) by compression of 0.25 g in a mold under a pressure of 50 MPa. The detection range for scattered radiation was 2θ = 5-40° at a speed of 2°/min. The crystallinity index (CrI) of the sample calculated as in Eq. 1 (Segal et al., 1959) using the height 200 peak (I_{200}, 2θ = 22.5°) and the minimum between 200 and 110 peaks (I_{am}, 2θ = 15.5°). I_{200} represent both the crystalline and the amorphous material, while I_{am} represents amorphous material.

$$CrI\ (\%) = (I_{200} – I_{am}/I_{200}) \times 100 \quad (1)$$

Fourier-transform infrared (FT-IR) spectroscopy

The powder RH-MCCwas place in sample holder and the surface of sample was characterized using Perkin-Elmer Spectrum 1000 Fourier transform infrared (FTIR) spectrophotometer. Each sample was scanned 64 times at a resolution of 4 cm^{-1} between 4000 and 650 cm^{-1}.

Nuclear magnetic resonance (NMR) spectroscopy

The sample was prepared as described by Diego et al., (2007), with slight modification. The RH-MCC sample was powder in a mortar and dried to a constant weight under vacuum. A 5 wt% suspension of the MCC in a IL-DMSO-d_6 was prepared by combining 100 mg of the sample, 1.6 g of [C_4mim] Cl and 300 mg of DMSO-d6 in a test tube followed by thorough vortexing. The mixture was then heated to 100 ˚C for 30 min with constant stirring, and subsequently filtered through glass wool to remove undissolved material. The resulting clear liquor was transferred to 5 mm NMR tubes. Proton-decoupled ^{13}C spectra were collected at 90 ˚C on a Bruker AVANCE 400 NMR spectrometer equipped with a 5 mm BBO probe operating at ^{13}C frequency of 100.61 MHz. A total of 20 000 scans was collected.

3. RESULTS AND DISCUSSION

Extraction of alpha cellulose

Effect of extraction method

Three extraction methods were used to pulp the rice husks agricultural residue. Table 1 shows that delignification was achieved to varying degrees. The yield of α-cellulose derived from the rice husks waste by the three pulping methods method range from 11.71 to 15.2%. The difference in the yields may be attributed to the different delignification power of the methods on the lignocellulosic

material (rice husks) resulting in varying compositions of α-cellulose and lignin. The results also revealed that the nitric acid pulping was most effective as it resulted in a very white pulp and the delignification was quite extensive since the pulp has low residual lignin content between 0.73%. However, the percent yield of pulp is low (11.71%). The low yield found with nitric acid pulping could be attributed to extensive degradation of the isolated cellulose by the reagents (especially nitric acid and sodium sulphite) employed in this method. It was important to obtain near lignin-free pulp, as high lignin value in the pulp will affect the stability of the derived MCC during shelf life. We observed in previous studies in our laboratory that MCC derived from pulps with high content of lignin often show discoloration or browning of the MCC powder during the shelf life.

The extraction of cellulose from rice husks sample using organosolvent method was incomplete and unsuccessful; sodium hydroxide extraction of cellulose was complete, but resulted in yellowish pulp, with high lignin content of 4.2%. However, Sun et al., (2005) reported complete pulping and extensive delignification using formic acid-acetic acid-water (30:60:10) mixture for the organosolv pulping of wheat straw. We observed that there seems be a correlation between the yellowish natures of some of the pulps (α-cellulose) with increased lignin content (0.73 and 4.2%). The near lignin-free pulp obtained by nitric acid method was used in acid hydrolysis process for the preparation of MCC, since we are interested in its suitability as pharmaceutical excipient.

Table 1 Effect of pulping method on the yield, nature and compositional propertiesof α-cellulose

Pulping methods	Yield (%)	Degree of pulping	Whiteness of pulp	Lignin content (%)	α-Cellulose content (%)
Sodium hydroxide	15.2 ± 1.4	Complete	Yellowish	4.2	42.7 ± 3.2
Nitric acid	11.7 ± 0.9	Complete	Very white	0.73 ± 0.1	37.0 ± 2.5
Organosolv	*	Incomplete	Yellowish	*	*

*Note: * Not determined because of incomplete pulping when some of the fibers are 'undegraded' or 'unaffected' by the reagents; number of extractions, n is 3.*

Pharmacopoeia critical attributes of MCC

The results of pharmacopoeial attributes of MCC derived from the rice husks residue are shown in Table 2.

Table 2 Some pharmacopoeial attributes of MCC derived from rice husk

Parameters	Values/Observations
Identification	Turns violet-blue with iodinated $ZnCl_2$
Organic impurities	Nil
Starch and dextrin	Nil
pH	6.4
Solubility (in ammoniacal solution of copper tetrammine	Complete and no residue
Water-soluble substance (%)	< 0.2
Loss on drying (%)	4.2 ± 0.4
Total ash (%)	0.1 ± 0.04

Note: Number of determinations n = 3.

Organoleptic properties

The organoleptic qualities of the RH-MCC were odorless, tasteless, white and granular in texture. This conform to the USP37-NF32 2014 description of organoleptic properties of MCC, which states that MCC/powdered cellulose (α-cellulose) ''occurs as a white or almost white, odorless and tasteless powder of various particle sizes, ranging from a free-flowing fine or granular dense powder, to a coarse, fluffy, non-flowing material''.

Cellulose identification

The MCC sample became violet-blue in color when it was reacted with iodinated zinc chloride solution, thus indicating the presence of cellulose in the samples.

Total ash

Total ash is a measure of the amount of material that remains after ignition and it consists of carbonates, phosphates, silicates and silica (Agrawal and Paridhavi, 2007). The value obtained for the total ash was very low, thus indicating that the MCC are almost free of inorganic compounds. The total ash value is of importance and is partly a reflection of the amount of care taken in the extraction procedure (Evans, 1989). The total ash value obtained for RH-MCC (see Table 2) is 0.26. This low value suggests that the high silica content associated with rice husk has been successfully removed by the nitric acid delignification method.

pH

The pH value is within the official value of 5 -7.5 (USP37-NF32 2014). Excessive pH values whether acidic or alkaline may results to poor shelf-life (Agrawal and Paridhavi, 2007). Loss on drying values (Table 2), are within the 6% maximum limit USP37-NF32 2014.

Water-soluble substances

The presence of water-soluble substances in the RH-MCC sample was less than 1% and fell within pharmacopoeia limits

Starch and dextrin

There was no color change when 0.05 M iodine was added to the MCC samples, indicating the absence of starch and dextrin from the samples. Thus, the MCC samples were free of these carbohydrates.

The foregoing official specifications, as contained in the pharmacopoeia, for microcrystalline cellulose emphasize tests for identity, quality and purity. To ensure excellent performance of MCC in drug products, it is required that other salient metrics be evaluated in the characterization protocol for MCC. Two of such critical metrics include crystallinity and spectroscopic determinations.

The crystallinity of cellulosic materials affects the practical performance of drug products. For instance, it influences properties such as compactibility and absorption of water. The latter influences the flowability during manufacturing processes, and the stability of the final drug products (Azubuike and Okhamafe, 2012). The X-ray diffraction pattern of the derived MCC is shown in Fig. 1. The diffractogramis similar to diffractograms for cellulose as reported in literature (El-Sakhawy and Hassan, 2007; Costal et al., 2015; Azubuike and Okhamafe, 2012; Alemdar and Sain, 2008). The diffraction peaks appearing at about 15.5° and 22.5˚ 2θ (due to 110 and 200 reflections, respectively), and the pattern are characteristic of cellulose II (Azubuike and Okhamafe, 2012; Segal et al., 1959). The crystallinity index of 50% was calculated for RH-MCC. This value for the derived MCC is lower than 58 - 69% reported for 11 branded MCCs that were evaluated by X-ray diffraction and FT-IR (Rowe et al., 1994). The low degree of crystallinity observed for the derived MCC samples could be due to the short duration (15 mins) of acid the hydrolysis of the α-cellulose.

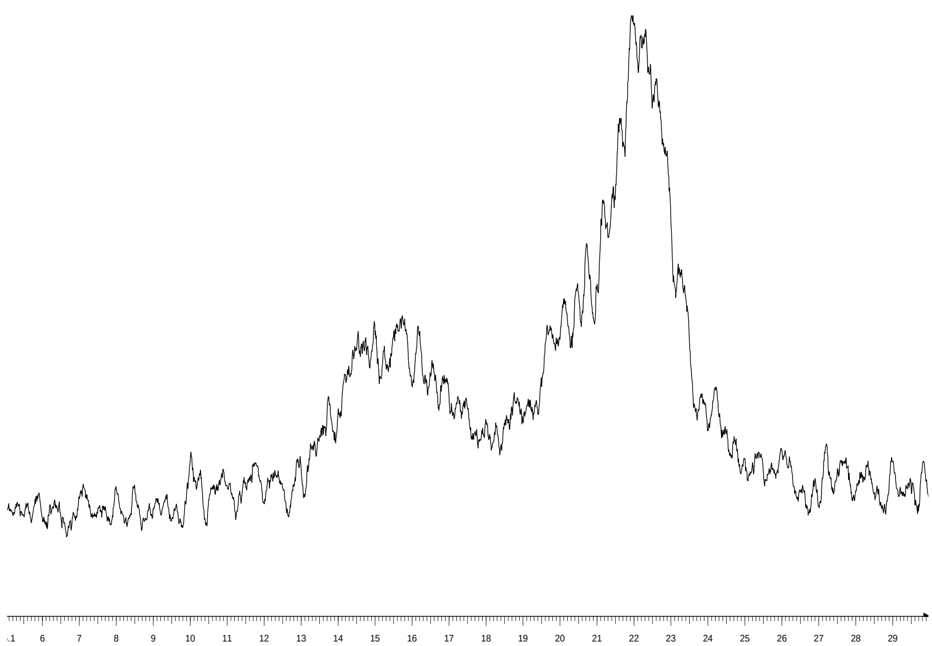

Figure 1 X-ray diffractogram of microcrystalline cellulose derived from rice husk

The FT-IR spectrum of the RH-MCC is shown in Fig 2. The spectrum did not show any absorption band at 1730, 1620, 1595 and 1512 cm^{-1}. These absorptions are usually characteristic of functional groups that are present in lignins of lignocellulosic materials prior to pulping (Viera et al., 2007). Thus, the absences of these absorption bands show, at least, that most of the lignin was removed during pulping and acid depolymerization process, or if present, it is in trace amounts that are undetectable by the FTIR.

Further analysis of the spectrum with reference to published data for cellulose (Grobe, 1989; Sun et al., 2005) shows several typical features of cellulose that include: (i) the characteristic intermolecular and intramolecular O—H stretching vibration band in the spectra which occurred at ~3330 cm^{-1}as well as C—H stretching at ~2890 cm (ii) the peaks at ~1425, and ~1315 cm^{-1} that are attributed to intermolecular hydrogen bonds at the C group and the O—H in plane bending vibration, respectively; (iii) an absorption band at ~1630 cm^{-1} is associated with the bending mode of the absorbed water, while the absorbance at ~1334 cm^{-1} originated from C—C and C—O skeletal vibrations; (iv) the peak at ~1160 cm^{-1} arose from C—O anti-symmetric bridge stretching and the C—O—C pyranose ring skeletal vibration gave a prominent band at ~1062 cm^{-1}; (v) the small sharp band at ~895 $cm^{-1}$is due to the glycosidic C_1—H deformation with ring vibration contribution and OH bendingthat is characteristic of β-glycosidic linkages between glucose in cellulose.

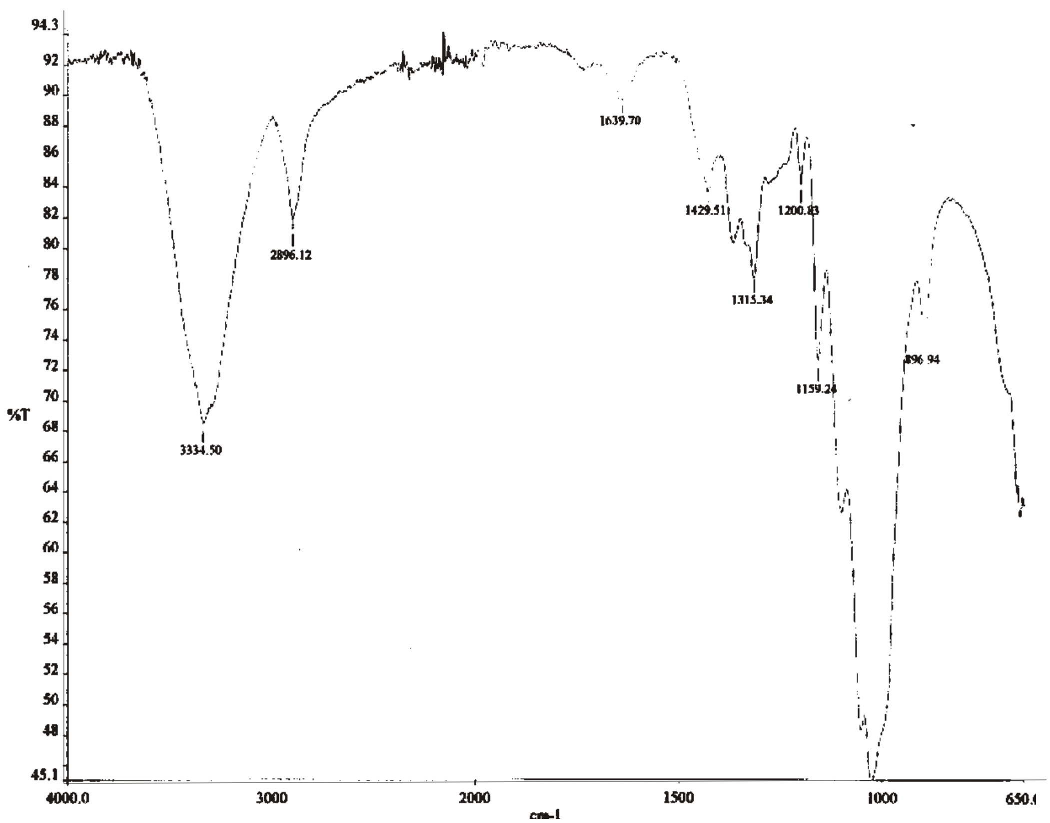

Figure 2 FTIR spectrum of microcrystalline cellulose derived from rice husk

NMR spectroscopy

In this determination, the use of small amounts of DMSO-d_6 as a co-solvent with the ionic liquid [C_4 mim]Cl has no appreciable effect on the solubility of cellulose, but rather it results in reducing the viscosity of the mixture. This is important so that the solubilized material could be analyzed directly with conventional ^{13}C NMR techniques. The ^{13}C spectrum of the liquor obtained after dissolving rice husks MCC for 1 h in [C_4 mim]Cl/ DMSO-d_6, is presented in Fig. 3. It shows the most unique features of the cellulose and of note are the signals at 103.1, 80.1, 76.2, 75.9, 74.7, and 61.0 ppm, all of which are well-resolved and correspond, respectively, to C-1, C-4, C-5, C-3, C-2, and C-6 carbons of cellulose (Diego et al., 2007). Also of note is the absence of a signal at ≈ 57 ppm in the spectrum; Diego et al., (2007) stated that the presence of this signal correlates with lignin aromatic OMe. Thus, its absence is shows that lignin is not present in the MCC in significant concentration. This observation seems consistent with the results obtained by FT-IR spectroscopy.^{13}C NMR spectroscopy has proved particularly useful for examining changes in the nature of cellulose subjected to degradation processes. In contrast to wet chemical analyses, NMR spectroscopy provides information about molecular orders in cellulose as well as description of any changes in the structure or content of hemicelluloses and lignin (Kim and Newman, 1995; Leary et al., 1987). ^{13}C NMR spectroscopy therefore complements the traditional chemical method of cellulose analysis. Additionally,

NMR technique has the advantages of minimal sample preparation, as well as simultaneously and quantitatively detecting signals from crystalline and less ordered states of cellulose (Larsson et al., 1997).

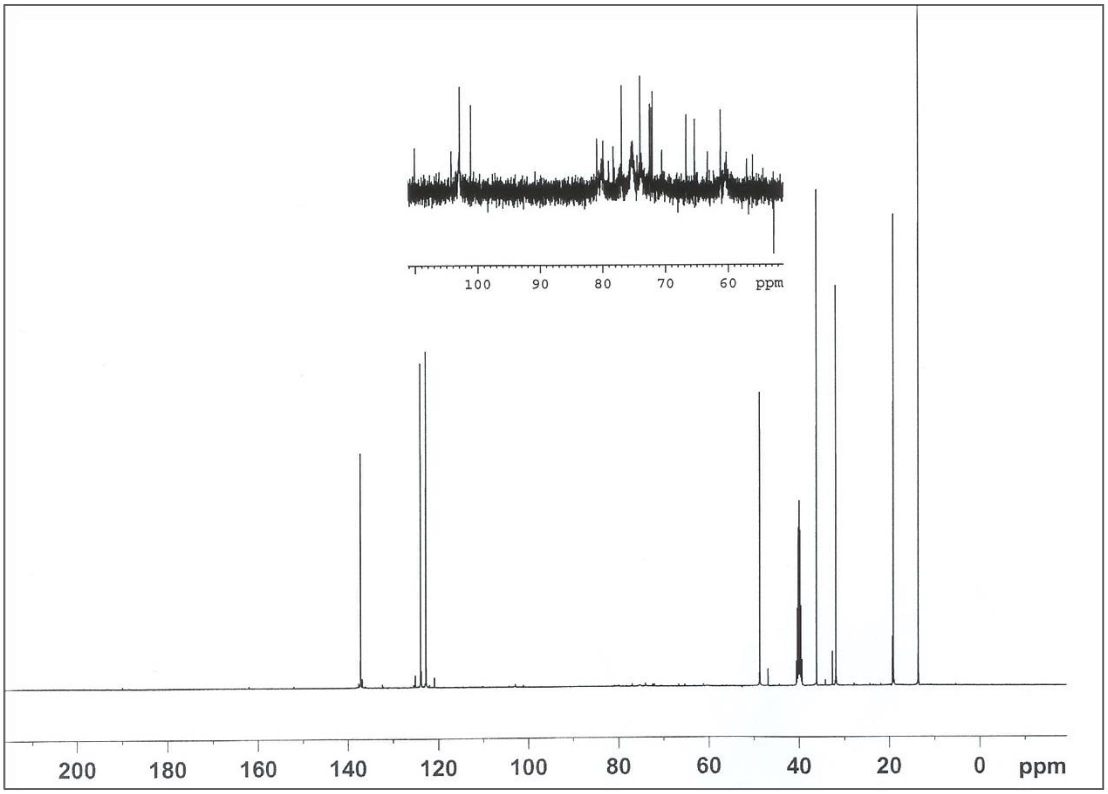

Figure 3 ^{13}C NMR spectrum of microcrystalline cellulose derived from rice husk

4. CONCLUSION

We demonstrated that rice husks biomass waste is a potential alternative feedstock for sourcing alpha cellulose; and it is technically feasible to obtain pharmaceutical grade microcrystalline cellulose from it, since the attributes evaluated are in accordance with official specifications (USP37-NF32 2014). Thus, microcrystalline cellulose derived from rice husk (RH-MCC) has potentials as a pharmaceutical excipient.

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

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